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Geographical Survey and Genetic Variation Studies of Few Chilly Varieties Collected from Different Regions of Karnataka, India

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ABSTRACT

Chillies are widely used throughout the world in the form of spice and are also used in making beverages and medicines. They are rich in vitamins, especially in vitamin A and C. Chillies contain lots of minerals like potassium, magnesium, and iron. They have been employed for pain relief as they are known to inhibit pain messengers and hence their extracts are used to alleviate the pain of arthritis, headaches, burns, and neuralgia. It is also claimed that they have the power to boost the immune system and lower cholesterol. They are also helpful in getting rid of gut parasites. In this regard we attempted to do a geographical survey about the chilly varieties and chilly growing states in India. We collected the local varieties of chillies available in Karnataka and performed genetic variation studies to understand their relationship at the genetic level. PCR analysis was done using the selected random amplified polymorphic DNA (RAPD) marker to identify the polymorphic loci between the genotypes taken. DNA Barcoding was done to validate the species using basic local alignment search tool (BLAST) and Clustal Omega, further dendrogram was constructed which guide the joining linkage rule of unweighted pair group average (UPGMA) and the genetic distance to compute from matrix table. The results obtained highlight the relationship between species of *Capsicum annum, Capsicum frutescens* which are closely related followed by *Capsicum pubescens* and *Capsicum baccatum* providing knowledge about its application in genetics and plant breeding in developing efficient hybrid variety.

Keywords: Chilly varieties, Dendrogram, DNA bar coding, Genetic variation, Geographical survey, Karnataka.

1. INTRODUCTION

Chilli is one of the most important vegetable crops for commercial use as well as spice. Christopher Columbus discovered chillies in the year 1493 which were then cultivated from 3500 B.C. Chilli originated from Mexico, and the secondary centers are present in Guatemala and Bulgaria.[1] Chilli is a member of the Solanaceae family which belongs to the Capsicum genus. It comprises approximately 20 species growing in tropical and subtropical regions of the world.[2] It consists of Capsicum species like *C. annum, C. baccatum, C. chinense, C. frutescens, C. pubenscens* etc. Chilli was introduced in India by Portuguese around 17th century which was then started incorporating into national cuisines.[3] India stands as the largest producer and consumer of chilli in the world. Chilli is also known as hot pepper, cayenne pepper, bell pepper, etc., and there are upto 400 different types of chillies found worldwide. The world's hottest chilli is cultivated in the hilly terrain of Assam, India which is known as "Naga Jolokia". Chilli is characterized by colour, aroma, taste, and pungency. This pungency is due to the alkaloid substance of capsaicinoids which is in the form of capsaicin and dihydrocapsaicin that makes upto 80–90%.[4] In India, it is cultivated in the states like Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Madhya Pradesh, Odisha, Rajasthan, West Bengal, etc. In India, Andhra Pradesh is dominated in the production of chilli contributes nearly 57% of production. The second-largest producer of chilli is Karnataka which contributes 12% of the total production. It is grown almost throughout the country and two crops are produced in kharif and rabi seasons in our country. Chilli grows best at 20–30°C where its growth and yield suffer when

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temperatures exceed 30°C or drops below 15°C for extended periods. This crop can be grown over a wide range of altitudes from sea level upto nearly 2100 meters. Chilli is very rich in vitamin C and A, antioxidant properties due to beta-carotinoids and minerals like folate, molybdenum, manganese, thiamine, and potassium.[5] Capsaicin compound increases the body's metabolism rate directly to burn down calories. India is the largest producer of red dried chilli in the world. Capsaicinoids have various applications among which its pharmaceutical applications include anti-obesity, anti-arthritic, analgesic, anti-microbial, and anticancerous properties.[6]

DNA Barcoding was discovered around 2003 and from then Barcode is allotted to almost all organisms to distinguish it from other species based on sequencing data. The sequence data consists of amplicons from a particular region of DNA produced through PCR techniques. Publicly accessible reference databases are constructed from DNA Barcoding results, which consist of specific DNA barcode sequences. Genetic variation is the differences in gene sequences between the individual species caused by mutations, random mating, random fertilization, and recombination. Random amplified polymorphic DNA (RAPD) markers are amplified DNA fragments with a single arbitrary primer in a PCR reaction, which synthesize DNA from sites to their matched primers. RAPD markers are extensively used for genotyping, phylogenetic analysis, and molecular selection.[7,8] These markers in molecular biology and DNA technology increase the possibilities of efficiency and efficacy in the diversity analysis of crop plants. In chilly, the genetic diversity analysis and varietal identification are done by using RAPD, SSR, ISSR, and AFLP.[9-12] So, in the present study, we have done a geographical survey about the chilly varieties and chilly growing states in India, collected few varieties of chilli plants, and analyzed the genetic variation among these varieties using the RAPD method, and authenticated the species using DNA Barcoding.

2. 2. MATERIALS AND METHODS

2.1. Geographical Survey

The survey was conducted to find out major Chilli growing states in India, their varieties, and state wise performance in area and production of Chilli in India.

2.2. Collection of Plants

The young leaves of the chilli variety were obtained from local chilli growing regions in Karnataka; in suitable seasons as chillies are grown in both the seasons Kharif (May to June) and Rabi (September to October). The samples were collected from places where they raise chilli crops with suitable conditions like frost-free and maintaining temperature range within 35°C–10°C. The optimum pH of the soil moisture content where the chillies are grown should be range from 6 to 6.5. The leaf samples were collected, surface-sterilized in distilled water, and stored for further use.

2.3. DNA Isolation

Genomic DNA was extracted from the young leaves of chilli by adopting the cetyltrimethylammonium bromide (CTAB) method. The leaf samples were crushed into fine powder in liquid nitrogen using mortar and pestle. Samples were taken in 1 mL of homogenizing buffer and incubated at 60°C for 30 minutes. After centrifugation (10 minutes, 10000 rpm), the supernatant was transferred to new clean tubes and the genomic DNA was extracted by adding an equal volume of chloroform: isoamylalcohol (24:1). The top layer was further transferred with chilled isopropanol and incubated overnight at -20°C. This solution was passed through DNA silica columns to obtain the purified form of DNA. Finally, 30 µL of genomic DNA was collected using pre-warmed elution buffer. 1 µL of RNase was added to the DNA solution and incubated at 37°C for 1hour to get rid of RNA impurities.

2.4. Amplification of Mitochondrial and Nuclear Gene

Amplification of genes from genomic DNA is done using polymerase chain reaction (PCR) mechanism wherein multiple copies of the specific gene are obtained. PCR was performed using template DNA (1 µL), forward primer (1 µL), reverse primer (1 µL), master mix (dNTP's, Taq polymerase, buffer MgCl2 – 12.5 µL), Nuclease free water (9.5 µL) and primers relevant for MatK and ITS 2 genes (Table 1). The program was set as shown in Table 1. The obtained PCR products wererun on a gel and subjected togel elution to get a purified form of template for Sanger sequencing.

2.5. Sanger Sequencing

Sanger sequencing is designed for the natural process of DNA replication, also referred to as dideoxy sequencing or chain termination method. This is based on the use of the addition of ddNTP's to the normal NTP's found in DNA. From the obtained template DNA primer, big dye terminator (BDT), dNTP's, and nuclease-free water were added to set the reaction. The BDT contains dNTP's, ddNTP's, Taq polymerases, fluorescent dye, and buffer. Followed by cycle sequencing the product was purified and subjected for capillary electrophoresis in a genetic analyzer.

2.6. Bioinformatics Analysis

The resulting file of DNA sequencing will be in the .ab1 format which can be viewed using software like FinchTV,

Table 1: Showing PCR program and primer sequences taken for study

	Temperature	*Time*	*Cycles*
Initial denaturation	940C	1 minute	1
Denaturation	940C	30 seconds	30
Annealing	550C	30 seconds	30
Extension	720C	1 minute	30
Final extension	720C	2 minutes	1
Primer sequences	MatK	MatKF-CCCRTYCATCTGGAAATCTTGGTTC MatKR-GCTRTRATAATGAGAAAGATTTCTGC	
	ITS2	ITS2F-ATGCGATACTTGGTGTGAAT ITS2R-GACGCTTCTCCAGACTACAAT	

SeqScanner, etc. The quality of the DNA sequence obtained can be visualized in electropherogram peaks. The sequencing data is analyzed using BLAST server swhich identifies closely related hits and further related sequences will be taken to understand the genetic relationship among different species. Clustal omega server is used to construct the phylogenic tree of the species.

2.7. Genetic variation studies

Using ITS2 primer the polymorphism between the species was identified by scoring the pattern and existence of bands in all the genotypes. Data values were entered into binary mixture as a discrete variable, where presence of band is 1 and absence of band is 0. This is done by the software PyElph 1.4 and matrix was subjected for further analysis. The dendogram was constructed by 0/1 matrix to infer the phylogeny and genetic relationships amongst them.

3. RESULTS AND DISCUSSION

The most widely chilli growing states in India are Andhra Pradesh, Maharashtra, Karnataka, and Tamil Nadu, which together constitute nearly 75 percent of the total area. Here Andhra Pradesh tops the list in dry chilli production followed by other states, where Karnataka takes fourth place. The major chilli growing states of India are shown in Figure 1 according to spices growing states of India. India is the only country that is rich in many varieties with different quality factors involved in chillies. Major varieties of chillies grown in the Indian states are Byadagi, Guntur, Kashmiri, etc as listed in Table 2. The same information of major varieties of chillies grown in Indian states is represented in pictorial form as shown in Figure 2. At the same, we have tried to estimate the area involved in the production of chilli throughout India. Figure 3 shows the last five years of data involving hectares of area utilized in the production of chilli in tons. An attempt was also done to analyze the production of chillies in the last 5 years in different states of India as

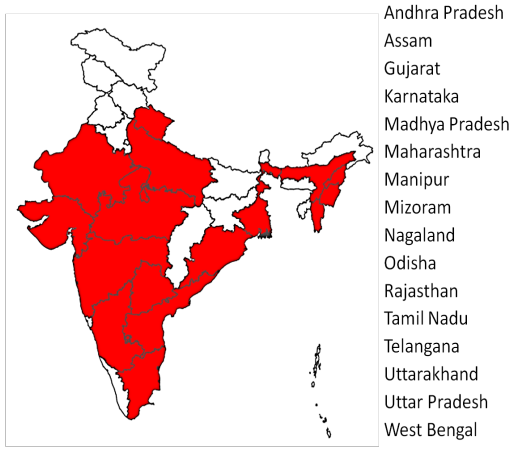

Fig. 1: Shows the major chilli growing states in India (www.indianspices.com)

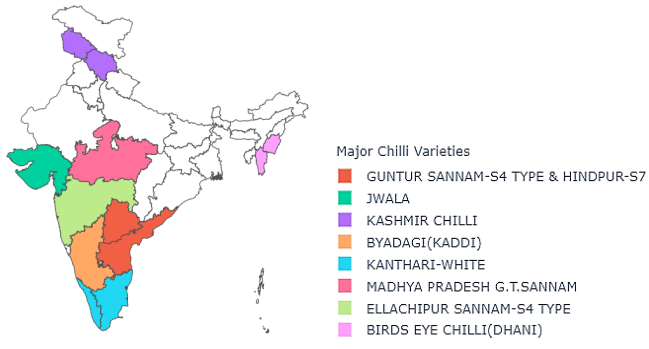

Fig. 2: Pictorial representation of major varieties of chillies grown in the Indian states

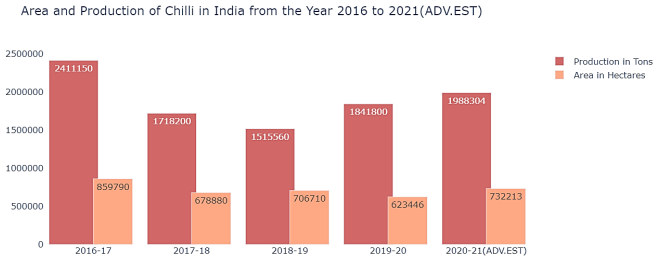

Fig. 3: Shows the recent area and production of chilli in India

shown in Figure 4. In all the cases Andhra Pradesh tops the list and Karnataka stands in fourth place. And this data is perthe data obtained by Indian spices.

Table 2: Highlighting major varieties of chillies grown in Indian states and their importanc

S.no	Variety name	Grown in states	Characteristics	Harvesting Season	ASTA Color Value	Capsaicin
1	Birds Eye Chilli (Dhani)	Mizoram and some areas of Manipur	Blood Red in Color, Highly Pungent	October to December	41.7	0.589%
2	Byadagi (Kaddi)	Karnataka	Red in Color with Less Pungency or Without Pungency	January to May	159.9	Negligible
3	Ellachipur Sannam-S4 Type	Maharashtra	Reddish in Color and Very Hot	September to December	70.4	0.2%
4	Guntur Sannam-S4 Type	Andhra Pradesh	Skin Thick, Hot and Red	December to May	32.11	0.226%
5	Hindpur-S7	Andhra Pradesh	Red in Color, Hot and Highly Pungent	December to March	33	0.24%
6	Jwala	Gujarat	Highly Pungent, Light Red in Color, Short and the Seeds are Compact	September to December	-	0.4%
7	Kanthari-White	Kerala and Tamil Nadu	Short and Ivory White in Color with High Pungency	Mainly grown as homestead crop	2.96	0.504%
8	Kashmir Chilli	Jammu & Kashmir, and Himachal Pradesh	Long, Fleshy and Deep Red in Color	November to February	54.10	0.325%
9	Madhya Pradesh G.T. Sannam	Madhya Pradesh	Red in Color and Pungent	January to March	-	-

ASTA: American Spice Trade Association

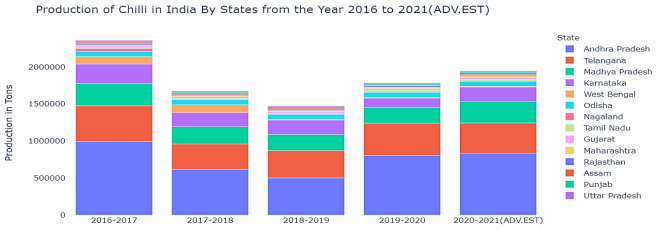

Fig. 4: Represents the last 5 years' data on the production of chilli in different states

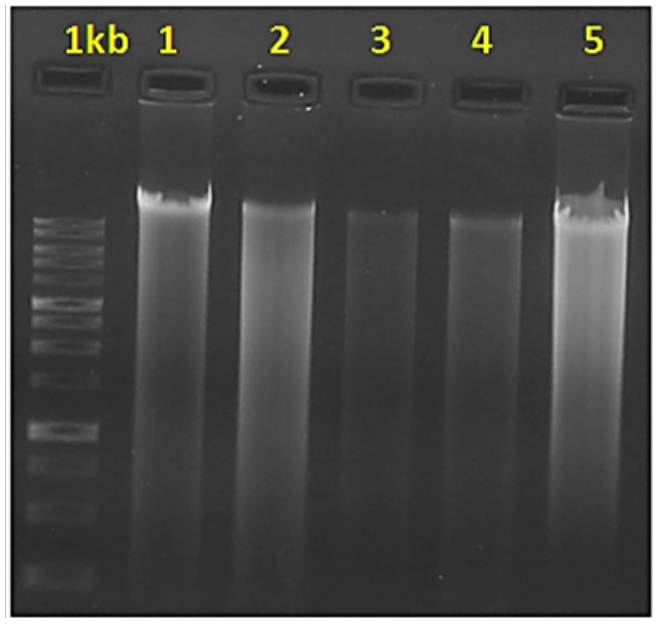

Fig. 5: Genomic DNA of different varieties of chilli, compared to 1KB ladder

In the case of molecular studies, we could collect five chilli species present around regions of Karnataka. Young leaves were subjected to genomic DNA isolation and their quality was checked through gel electrophoresis as shown in Figure 5. The target genes MatK and ITS2 were amplified using the PCR program and products were collected as shown in Figure 6. The sequences obtained from capillary electrophoresis were subjected to the national center for biotechnology information and the basic local alignment search tool (NCBI BLAST) to identify unknown sequences. Table 3 shows the identified species as *Capsicum pubescens, Capsicum frutescens, Capsicum baccatum,* and *Capsicum annuum* two varieties. The phylogenetic tree is constructed with obtained species relating with nearby 10 species to understand their evolutionary relationshipas shown in Figure 7. In genetic variation studies, the primer ITS2 was used to check the polymorphism between the species. The banding pattern obtained was analyzed on the gel using PyElph 1.4 to see the lanes, bands, and matching bands. Based on this binary matrix was constructed which was thus used to construct a dendrogram. Figure 8 shows the polymorphic bands obtained by five chilli species using the

Table 3: BLAST results identifying the species of chilli varieties taken for study

Sl. No	Sample	Gene	Description hit	Query cover	E value	Percentage identity	Accession number
1	BD	MatK	Capsicum pubescens chloroplast, complete genome	95%	0.0	97.85%	NC_039694.1
2	GU	MatK	Capsicum frutescensmaturase K gene, partial cds; chloroplast	94%	0.0	96.44%	MN528466.1
3	RB	MatK	Capsicum sp. HO-2018B T4/ Yokohama/JPN/2017 chloroplast matK gene for maturase K, partial cds	98%	0.0	97.09%	LC385926.1
4	SY	MatK	Capsicum annuum NIAS:JP 124339 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	98%	8e-166	90.30%	LC510559.1
5	5531	MatK	Capsicum frutescens maturase K gene, partial cds; chloroplast	94%	0.0	99.04%	MN528466.1
6	BD	ITS 2	Capsicum baccatum var. pendulum isolate 35 maturase K (matK) gene, complete cds; chloroplast	83%	8e-04	96.97%	EF537317.1
7	GU	ITS 2	Capsicum annuum NIAS:JP 82498 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	70%	1e-168	99.70%	LC510557.1
8	RB	ITS 2	Capsicum annuum voucher 13 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	75%	2e-167	99.40%	KY932213.1
9	SY	ITS 2	Capsicum annuum NIAS:JP 124339 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	98%	8e-166	90.30%	LC510559.1
10	5531	ITS 2	Capsicum pubescensUSDA:GRIN:Grif 1614 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, clone: Grif1614, sub_clone: Grif1614_1	13%	2e-12	90.77%	LC5105595.1

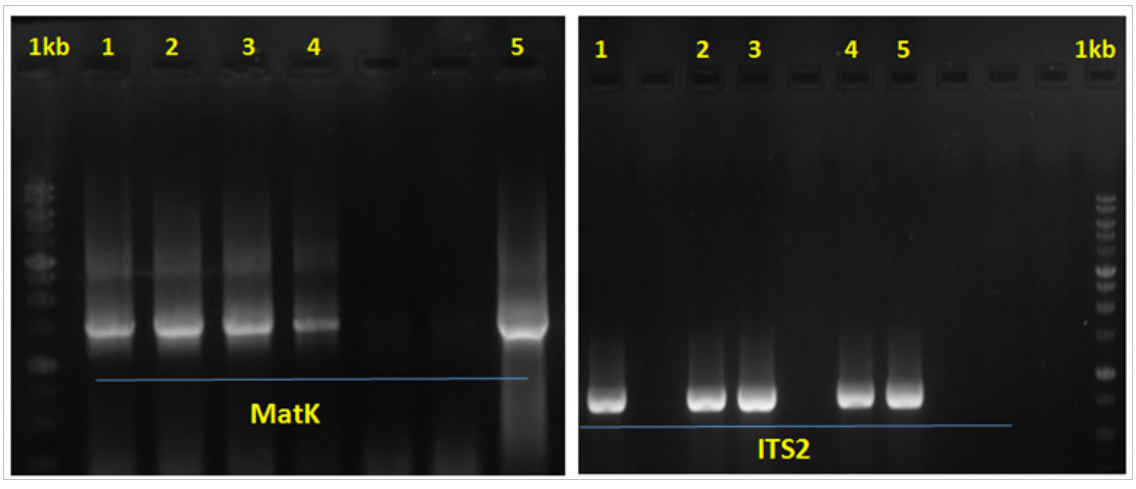

Fig. 6: Showing amplified MatK and ITS2 genes from genomic DNA

software. Distance matrix data was converted to UPGMA tree which shows the genetic relationship between the chilli species. According to the tree in Figure 9, it says that *Capsicum annum* and *Capsicum frutescens* are closely related followed by *Capsicum pubescens* and then *Capsicum baccatum.*

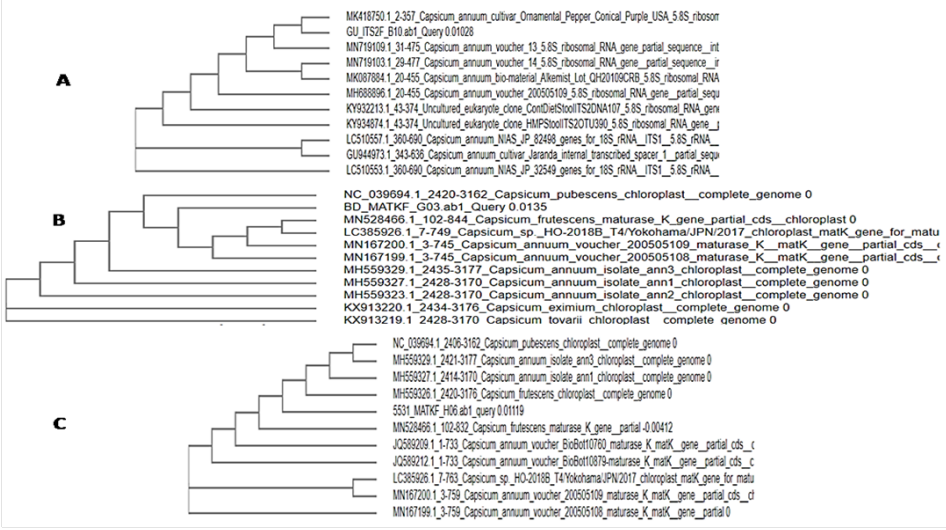

Fig. 7: Representing phylogenetic data obtained from each entry to understand their evolutionary relationship

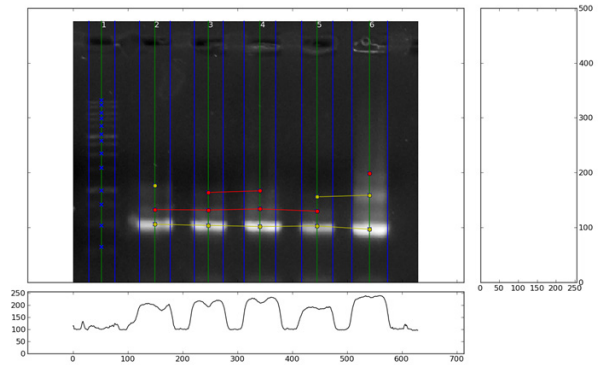

Fig. 8: Polymorphic bands obtained by five chilli species as seen in pyelph 1.4

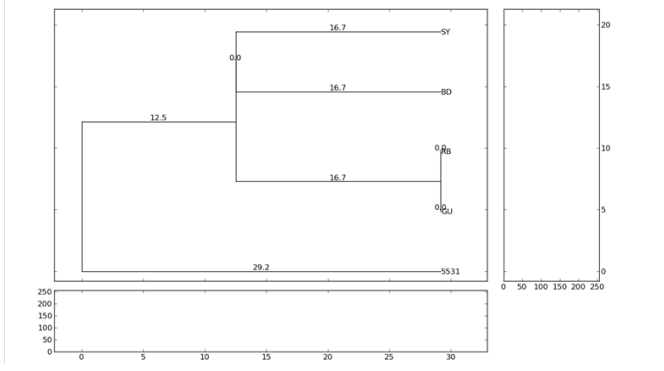

Fig. 9: UPGMA tree showing the genetic relationship between the chilli species

Chilli is being cultivated in almost all the states in India where Andhra Pradesh stands as the largest producer accounting for 50% of total chilli output in the country. Karnataka also contributes to about 10–15% of total production in the country.[13] *Capsicum species* are one of the most important vegetables because of their double use as vegetable and spice grown in two commercial stages like both unripe and fully ripe which contributes to their use in many recipes and fast diffusion. They contain many bioactive compounds essential to provide high added-value to cultivars and for organic markets though knowledge about organic cultivation on *Capsicum* fruit is very much scarce. A large genotypic variation was recorded within each ripening stage and growing condition for the already studied research traits. Significant genotype versus ripening stage and growing conditions versus ripening stage interactions suggested that the magnitude of increase with ripening depends on the accession and growing conditions. But in contrast, no differences were seen between growing conditions for carotenoids and differences depend on the genotype factor. Ana *et al*., (2018) encompassed a huge genetic variation among cultivated species like *C. annuum* and its relatives, members of *annuum* complex, *C. chinense a*nd *C. frutescens*.[14] In addition, other species like *C. baccatum* and *C. pubescens*are profusely cultivated and consumed. Flavonoids like quercetin and luteolin are the most abundant group in *Capsicum* fruits and are excellent antitumoral, antioxidant, and antiviral agents that are attributed to their metabolites.[15,16] The diversity of colors among fully ripe fruits of *Capsicum* is also due to the combination of up to more than thirty carotenoids.[17] And the success of DNA barcoding lies in the distinct identification of the clusters in the phylogenetic analysis.[18,19] Barcoding tools also provide authentication to any plant species and hence support conservation measures of the plants in several ways. In this regard species, delimitation and identification act as the first critical step inaccurate assessment of distribution, their population abundance, and threats caused to target species.[20] Thus, barcoding can speed up the identification process of collections which includes sterile material,and also enhances the knowledge on species distributions and their abundance.

4. CONCLUSION

The present study was carried out to identify different varieties of chilli species grown in India and their geographical locations. This indicatedto assess the genetic diversity of chilli varieties present in Karnataka.Understanding of these traits that are of economic importance can be further used for improvement programs of genetic potential in chilli species.Cluster analysis stands to be an effectivemethod in grouping the species fromdifferent regions which will facilitate the conservation, management, and utilization ofgenetic traits by selecting accessions having good economic traits. The potential of integrating polymorphism and PCR into programs of plant improvement is enormous and acts as major area of application in the mere future.

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